Best Practices

For Germplasm Management

A New Approach for Achieving Genebank Standards

T. Calles, M.E. Dulloo, J.M.M. Engels and I. Van den houwe

FUTURE HARVEST

Contents

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Acronyms and Abbreviations

CBD	Convention of Biological Diversity
CGIAR	Consultative Group on International Agricultural Research
CIAT	Centro Internacional de Agricultura Tropical
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo
FAO	Food and Agriculture Organization of the United Nations
IBPGR	International Board of Plant Genetic Resources
ICARDA	International Center for Agricultural Research in the Dry Areas
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IITA	International Institute of Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung
IRRI	International Rice Research Institute
ITPGRFA	International Treaty of Plant Genetic Resources for Food and Agriculture
NBPGR	Indian National Bureau of Plant Genetic Resources
NCGR	The US National Clonal Germplasm Repository
NCGRP	The US National Center for Genetic Resources Preservation
USDA	United States Department of Agriculture
USDA-ARS	Agricultural Research Service of the USDA
GRC	Genetic Resources Center
HEPA	High Efficiency Particle-removal Air system
IRGCIS	The International Rice Genebank Collection Information System
KB	Karnal bunt
MOU	Memorandum of Understanding
MTA	Material Transfer Agreement
MZBANK	Maize Genebank Information Database System
PEQIA	Post-Entry Quarantine Isolation Area
RH	Relative Humidity
SHU	Seed Health Unit
SIDU	CIMMYT Seed Inspection and Distribution Unit
SINGER	The System-wide Information Network for Genetic Resources
sMTA	Standard Material Transfer Agreement
WGBS	Wheat Germplasm Bank System

1. Introduction

To ensure the genetic integrity of the germplasm kept in genebanks, the conserved material should be maintained to the highest standards possible over a prolonged period of time. The only way to achieve that is setting standards based on current best practices and available technologies which will guarantee the conservation of the collection over longest possible time and thus increase the necessary intervals of seed regeneration (Dulloo and Engels, 2003).

The first standards for genebank was drawn in 1975 by the Panel of Experts on Plant Exploration and Introduction who made the first recommendations on preferred and acceptable standards (Dulloo and Engels, 2003). Ten years later, the IBPGR Advisory Committee on Seed Storage established standards not only for ensuring the safety of plant genetic resources but also the safety of staff working in the genebank (IBPGR, 1985). In 1992 the FAO/IBPGR Experts Consultation Group on Genebank Standards refined the standards established 1985, adapting them to the advances made in the seed storage technology (FAO/IPGRI, 1994). At the present time, those international standards are used in national, regional and international genebanks.

There are several problems in setting standards and it is debatable whether generic standards are the best way to achieve the good management required for ensuring the genetic integrity of the germplasm kept in the global genebank network. Since seed conservation technology is constantly improving, standards for genebank management should be updated in line with the new technologies which have been improved; however, it is not occurred due to different difficulties. Additionally, it has been scientifically proven that different seeds have different storage behavior. The aforementioned reasons give the ground to conclude that generic genebank standards *per se* may be not so helpful for achieving an effective management of germplasm collections.

Plant genetic resources conservation of vegetatively propagated and 'recalcitrant' species has been significantly improved in the last decade. Nowadays, many field genebanks have developed *in vitro* active collections to conserve these species (Engelmann, 1999). However, these developments took place without the benefit of generic *in vitro* genebank standards being available. This lack of standards hampers the harmonization of the global genebank network and difficult networking. Currently, there is a need for producing generic genebank standards for *in vitro* collection management in order to harmonize global genebank network.

There is no doubt that the term 'best practices' can create discrepancy among researchers and genebank curators. The main conflicts that may create the term are that many people will ask themselves which is the difference between generic genebank standards and best practices? Who decided that these are the best practices? And based on which parameters they selected the best practices? Nevertheless, the purpose of this research is not other than share the information of well established research centers, in order help with the harmonization of the global genebank network. Moreover, the provided information on best practices may help small national genebank to achieve genebank standards and thus safeguarding their genetic resources.

A complementary and novel approach is proposed for improving genebank management which consists in combining generic genebank standards and best practices. This approach can be very helpful because: on one hand, generic genebank standards provided the scientific background needed to properly conserve the germplasm and on the other hand, best practices gives the technical knowledge on how to reach those standards

The best practices presented were neither selected for a scientific board of experts nor based on specific parameters. However, they were compiled from different well established and recognized institutions (i.e. CGIAR Genebanks) which are constantly researching on effective ways of improving management of germplasm collection. Moreover, these institutions are staffed by worldwide experts in different areas regarding with genebank management. These institutions have gained considerable expertise in germplasm conservation and they have been able to develop procedures for effective management of germplasm collections. In summary, the aforementioned reasons give sufficient grounds to conclude that this novel approach shall be helpful for harmonizing global genebank network and ensuring the security of the germplasm collections.

Generic genebank standards and the best practices were compiled from available literature. Mainly genebank operations manuals of different CGIAR Genebanks were used to colleted best practices. Additionally, interaction with some genebank curators was done to gather information not covered by the operations manuals and to plan the research strategy. Best practices were compiled for the following crops: rice, pearl millet, maize, chickpea, wheat, pigeonpea, and *Musa*. The criteria used for selecting the crops were: (a) availability of operations manual for the crop and (b) the crop should be listed in the Annex 1 of the International Treaty.

This research could not cover a wider number of crops because there were not operations manuals available that cover the rest of the Annex 1 crops. There are only three CGIAR Centers that have operations manuals which are CIMMYT, ICRISAT, and IRRI. Probably, most genebanks have unpublished operations manuals for internal used; these genebanks should put some effort for publishing the manual, in order to make available the information produced by them.

The objectives of this research were the following: (a) to compile information for developing the first generic *in vitro* genebank standards and the best practices for *Musa* species. This represents a mile stone because for *in vitro* little management information is neither available nor complied in a comprehensive manner and (b) to collate generic seed genebank standards and the best practices for the following crops: rice, pearl millet, maize, chickpea, wheat, and pigeonpea. Further research efforts are needed to complete the rest of the crop listed in the Annex 1 of the IT.

The ultimate goal of this research is compiled as much information as possible, in order to help genebank to achieve an affective management of germplasm collection and thus ensuring the genetic integrity of more than six million of germplasm accessions kept in the global genebank network.

2. Generic Seed Genebank Standards

2.1 Overview

The primary objective of setting generic genebank standards is to ensure that genebank operations (or practices) are carried out in an effective and efficient manner. To this effect, there are four conservation objectives that cover the full spectrum of genebank/germplasm management:

Ensuring security
Maintaining genetic integrity
Ensuring availability
Providing information

For each of these objectives, generic standards will be provided; in addition, a set of crop specific best practices will be collated. This is a complementary approach where generic standards provide the scientific background needed to properly conserve the germplasm and best practices for specific crops to give the technical knowledge on how to reach those standards.

2.2 Ensuring Security

It refers to the security of the genebank structure itself (i.e. its physical security) and to the safety of its germplasm (i.e. the maintenance of viability) which together will ensure the long term conservation of the entire collection.

2.2.1 Physical Security

To ensure the physical security of the collections, these generic standards are regard as essential prerequisite for achieving the objective:

2.2.1.1 Safety Duplication: Replication is required to ensure that accessions conserved in the genebank are safely duplicated in another location. A representative duplicate of an accession that is conserved for the long-term should also be stored in another genebank, under the 'black-box' arrangement. The latter means that the recipient genebank has the sole responsibility of storing the duplicated accessions in their genebank and does not have any other responsibility for that material. It is suggested that the safety duplicated accessions should be stored in another country, preferably in another continent, and under equal or better storage conditions than the "original" accessions. Additionally, the material may be conserved in 'Svalbard', as a safety back-up, i.e. second level of safety duplication.

2.2.1.2 Structure: The premises should be built to withstand most of likely natural disasters like hurricanes, cyclones, earthquakes, flooding, etc. The storage facilities should also be protected with fences, alarm systems, security doors and any other systems that help to shield the genebank against burglars. Additionally, an early detection fire system should be in place and the premises should be equipped with fire isolation doors and extinguisher equipment. Doors of the cold chambers should have a mechanism that allows to open the door from inside, so as to ensure security of genebank personnel.

2.2.1.3 Equipment: The genebank should be equipped with an emergency electrical generator which provides automatically back-up power to the storage rooms, essential genebank lighting, monitoring devices, and access locks during electrical power failures. Monitoring devices for temperature should be available in the storage and drying rooms to track the actual parameters against time. It is also recommended that the rooms are provided with a back-up compressor which will keep temperature down in case of main cooling system fails. Refrigeration, electrical generator and any other back-up systems must be run alternately every month to ensure that they continue to work properly.

2.2.1.4 Contingency Plans: An emergency plan for the genebank should be implemented; it ought to describe actions and measures to be taken when any possible disaster occurs; in addition, genebank staff should be trained to confront any emergency situation. A full risk assessment of the genebank operation should be carried out and published.

2.2.2 Maintenance of Viability

This section refers to the maintenance of the longevity of the seeds in storage. A high initial viability is the most important pre-condition for achieving the longest lifespan of seed accessions in storage, hence maximum efforts need to be taken to ensure that seeds to be stored have the highest possible viability. Besides optimum growing conditions when regenerating the accessions as well as the most efficient management of the preparatory steps before storage, the following three generic standards are critical in the maintenance of the viability of stored material:

2.2.2.1 Storage Conditions: Storage temperature and seed moisture content are the two most important factors that influence the longevity of stored seeds. It has been scientifically proven that the optimum storage temperature for maximum longevity is -18 °C (or lower) and that the seed moisture content should be between 3-7 %, depending upon the species stored.

2.2.2.2 Initial Viability: As mentioned above, the initial seed viability is strongly correlated with the lifespan of seeds in storage and provides the baseline for subsequent viability monitoring. The target will be to achieve an as high as possible initial viability and to determine this as accurately as possible for all accessions. Problems in determining the initial seed viability that are related to dormancy and hardseededness should be taken into account and managed properly.

2.2.2.3 Viability Monitoring: A gradual decrease of seed viability naturally occurs during storage which accelerates when reaching a certain viability threshold and may vary from species to species. Therefore, accessions stored in a genebank should be monitored to avoid excessive deterioration of seeds and, thus a possible loss of genetic diversity. The first monitoring test should be performed latest after 10 years of storage for accessions with a high initial viability which will vary upon the species dealt with and will be far above to the threshold for regeneration. It is only applied when the collection is maintained under standard storage conditions. Wild relatives and species known for having low or erratic initial viability and/or a known poor storage lifespan should be tested latest after five years. Dates for the

second and subsequent tests should be based on curator's experience as well as on the results of the preceding viability test(s).

The accession must be regenerated when: (a) seed viability drops below the threshold of 85 % and/or (b) numbers of seeds descend further down than 1,500.

2.3 Maintaining Genetic Integrity

Maintaining the genetic integrity of an accession can be achieved by minimizing genetic drift which may occur predominantly during the process of regeneration due to too small numbers of individuals being planted, sub-optimal pollination and/or the introgression of alleles from other accessions or commercial crops or crop wild relatives. The following generic standards are required for achieving the objectives of maintaining genetic integrity.

For further guidance on maintaining genetic integrity during regeneration, please refers to "Regeneration of Accession in Seed Collections: A Decision Guide" (Sackville Hamilton and Chorlton, 1997).

2.3.1 Minimum Sample Size for Regeneration: Each sub-sample to be used in regeneration should possess an adequate number of seeds to ensure maintaining the original frequency distribution of all alleles at all loci. It is critically important to avoid genetic drift caused by population bottlenecks. It is also crucial to ensure an adequate production of seeds during regeneration for replenishment of stocks being stored as safety duplicates, as active and/or as base collections. The optimum sample size will depend on the breeding system of a given species (e.g. out-breeding species require a higher number of plants for regeneration than in-breeding species). As a generic standard, FAO/IPGRI recommends to use 100 plants or more for regeneration.

2.3.2 Pollination Controls: Unless the species is an obligate apomict or self-pollinating species, appropriate pollination control measures should be implemented to ensure the genetic integrity of the accession. Control measures to be used will depend on the species and include: (a) preventing pollination by alien pollen through proper isolation, (b) ensuring pollination is fully effective by use of relevant pollinators, use of fan for wind-pollinating species, etc., (c) minimizing differential contribution of male gametes by artificial pollination, and (d) ensuring appropriate female-male pairing by isolation, manual pollination, etc.

2.3.3 Appropriate Regeneration Environment: Ideally, regeneration of germplasm accessions should be carried out in the same or similar environments from where the germplasm accession originated. It is understood that genetic drift may increase or yield and seed quality reduce if accessions are regenerated under conditions that differ significantly from the ecology of the collecting site. Genebanks need to have either a network of sites in different agro-ecological zones or conduct the regeneration in controlled environments (e.g. greenhouses, screen cages, etc.) that meet the environmental requirements for the accession in question.

2.4 Ensuring Availability

An important objective of conservation efforts is to facilitate the effective utilization of germplasm accessions by researchers, breeders and farmers. Thus, ensuring the ready availability of stored germplasm is an important principle. It refers to the ability of genebanks to supply and distribute the stored germplasm, together with any associated information, to users. Aspects that can affect the availability include: (a) policies, (b) seed stock, (c) health status of accessions, and (d) distribution quantity.

2.4.1 Policies: The availability of germplasm accessions will depend on the legal status of the individual germplasm accessions. There are four basic categories of germplasm in relation to its legal status:

a. accessions of crops and crops complexes that are listed in Annex 1 of the International Treaty (IT) of PGRFA is governed by the Treaty; access to these genetic resources will be provided under a standard material transfer agreement (sMTA) approved by the IT's Governing Body,
b. accessions that have not been listed in Annex 1 of the IT and have been acquired after the entering into force of the Convention on Biological Diversity at January 1st, 1994. Access to these genetic resources will be provided imposing on the user the terms and conditions set by the country of origin of said resources (which could match those of the IT or even be no conditions),
c. all other genetic resources, that have not been listed in Annex 1 of the IT and have been acquired before January 1st, 1994, which the CGIAR hopes to make available under terms and conditions identical to those stated in the standard MTA of the IT (this is subject to the approval of the Governing Body of the IT) and,
d. material that is improved or is under development, which the CGIAR also hopes to make available under conditions that are the same as, or at least broadly similar to, those of the sMTA.

For further guidance on germplasm exchange policies, please refer to Fowler et al. (2003) or Barton and Siebeck (1994).

2.4.2 Seed Stock: Genebank should ensure to keep at all times adequate amounts of seed for each accession in order to be able to supply to requestors, to monitor the viability as well as to carry out regeneration. It is difficult to set a generic standard for the number of seeds that should be conserved for all species; this will depend on the reproductive biology of the species (i.e. self-pollinating, cross-pollinating) as well as on the demand of individual accessions. As a generic standard, FAO/IPGRI suggests a minimum of 1,500-2,000 seeds per accession; nevertheless, more seeds may be necessary in the case of genetically heterogeneous accessions.

For further guidance on how calculate the number of seeds per accession required for the base and active collections, please refers to Sackville Hamilton and Chorlton (1997).

2.4.3 Health Status of Accession: Disease infected accessions may decrease the ultimate availability of the germplasm material. Collection managers should aim to store disease free germplasm, whenever possible, as accessions that are being distributed should be pest and disease free (at least of quarantine pests and diseases) in order to comply with national and international quarantine regulations.

For further information about crop specific protocols for germplasm movement, refer to the IPGRI/FAO's "Technical Guidelines for the Safe Movement of Germplasm" which can be downloaded at:
http://www.ipgri.cgiar.org/system/page.asp?frame=publications/indexpub.htm

2.4.4 Distribution Quantity: It is recommended that genebank managers distribute germplasm samples with an adequate number of seeds per accession in order to adequately cover the genetic diversity present in the respective accessions and with viability exceeding 85 %. There is not a generic standard for the quantity of seeds that should be distributed per accession as this will depend on the species in question. However, it is recommended to provide at least 100 seeds per accession of cross-pollinating species; and 50 seeds for a genetically homogeneous accession.

2.5 Providing Information

The lack of adequate information on a given accession may well decrease the value of that accession to the end-user. The information on individual accessions should be as complete as possible in order to contribute to the effective conservation and use of genetic resources. This applies for example in the identification of duplicates and/or the selection of accessions with desirable characteristics/traits. To achieve this, a genebank should have a management system in place that allows optimizing management of the collections as well as providing access to valuable information about the material to end-users (e.g. breeders, researchers, etc.).

2.5.1 Genebank Management System: The daily management of a genebank requires access to accurate information on the germplasm conserved. These operations cover a wide range of activities from acquisition, registration, seed viability, seed health, regeneration, evaluation as well as distribution to end-users. To achieve this, the system should allow staff members to access information about the following types of data: (a) passport, (b) management, (c) characterization, (d) evaluation, (e) mode of reproduction, and (f) seed distribution. Such management system differs a lot from a conventional information system that attempts to capture and manage information. A genebank management system requires an effective decision-making system based on daily operation workflows.

2.5.2 System for Information Exchange: In order to facilitate access and use of germplasm by the end-users, it is required that the most valuable information on the origin, characteristics and performance of the material is made readily available. Establishing such information exchange mechanism first requires a better understanding of the potential users and their needs. The management system should be structured taking into consideration the following aspects:

a. It should be developed taking into consideration the needs of the possible different users (e.g. farmers, researchers, breeders, etc.).
b. Genebank knowledge about the collection should be included in the management system (e.g. breaking dormancy methods, important traits, recommended subsets for particular traits combination, etc.).
c. The system should be able to monitor germplasm flow and obtain users feedback.

The easiest way to give users access to the relevant information may be through the Internet. However, it should not be forgotten that access to the Internet is not reliable in some countries or not available at all; for these reasons, critical information of genebank operations should be also published in germplasm catalogs, articles, publications, or operations manuals.

3. Best Practices for Rice: An Example of IRRI's Approach[1]

3.1 Overview

Rice is one of the world's most important food crops. It is a valuable crop, not only because almost half of the global population depend on it but also in the view of the fact that rice is the single most important income source for the rural poor. During decades, rice genetic resources have been used to increase the productivity of the rice. The crop comprises two species, *Oryza sativa* (Asian rice) and *O. glaberrima* (African rice), and 22 distantly related wild species of rice.

There is a real need for conservation of rice genetic resources due to traditional varieties and wild species are being lost through genetic erosion. Farmers adopt new varieties and eventually lose those that they have been nurtured for generations. Additionally, wild species are constantly under threat since their natural habitats have been severally modified or destroyed by humans. Rice's genetic diversity is key element of any future rice improvement owing to the fact that a broad genetic base is required to cope with the biotic and abiotic stresses that faces rice production.

The International Rice Research Institute (IRRI) was found in 1960 with the mission of safeguard genetic diversity of rice. In 1962, the institution started collection, conservation and characterization activities aiming to find sustainable ways to improve the well-being of present and future generations of poor rice farmers and consumers while at the same time protecting rice genetic diversity. At the present, IRRI genebank holds over 100,000 accessions.

3.2 Ensuring Security

3.2.1 Physical Security

3.2.1.1 Safety Duplication: As a general rule, all accessions holded by IRRI have a safety duplicate sample stored elsewhere. IRRI has signed, in 1993, an agreement with the United States Department of Agriculture, Agricultural Research Service (USDA-ARS); under the terms of this arrangement, once a year, safety duplicate samples are shipped for long term conservation to the US National Center for Genetic Resources Preservation, in Fort Collins, Colorado, USA.

The security duplicate or 'black-box' is prepared in small aluminum foil packets, containing 20 g of seeds for *Oryza sativa* and *O. glaberrima* and 50 seeds for wild rice accessions.

3.2.1.2 Structure: *The operations manual does not provide any information about the premises*.

3.2.1.3 Equipment: The genebank has a separate standby generator aside from the generator serving the whole IRRI and a back up compressor. The drying room and storage areas have a digital monitor system which is connected to a chart recorder to note the fluctuation in temperature and relative humidity during night time. In the drying room, an alarm signal is connected to relative humidity and

[1] Technical information has been obtained from International Rice Genebank Operations Manual (IRRI, 2002).

temperature sensors. In the cold storage rooms, there is a door interlocks system that ensures warm air does not flow into the storage room. Moreover, the base collection has a red light signal for warning the staff when the door is open. The back up refrigeration system is running alternately on monthly basis to ensure that it remains in good working order. The refrigeration has a time-switch defrost cycle to maintain the equipment in good shape.

3.2.1.4 Contingency Plans: *The manual does not provide information regarding emergency plans*.

3.2.2 Maintenance of Viability

3.2.2.1 Storage Conditions: IRRI maintain its active collection stored at +2 °C and the base collection is conserved at -20 °C. The seeds are dried in a drying room at 15 °C and 15 % RH until they reach a moisture content of 6 %.

3.2.2.2 Initial Viability: The methodology used by IRRI is as follow, the seeds are placed in the oven set at 50 °C for five days to break the dormancy (only applicable for *Oryza sativa* and *O. glaberrima*), then seeds should equilibrate at room temperature (28-39 °C) for 2 to 3 days prior germination. Seeds are sowed in moist paper towels and place in a germination chamber set at 30/20 °C alternating temperature on 12/12 h duration and in a 12/12 light/dark condition with 99 % of relative humidity. The count of normal and abnormal seedling is carried out 7 days after germination and a second reading at 14 days, when necessary.

For wild rice, only twenty seeds are germinated after breaking dormancy. Strong dormancy in some seeds has been observed and requires combination of dormancy treatments (see Naredo *et al*., 1998).

Only *Oryza sativa* and *O. glaberrima* materials with viability exceeding 90 % are packet for long term conservation, except for some materials which exhibited consistently lower viability potential, such as japonica, glutinous, and large seeded materials which have 85 % viability cut off.

3.2.2.3 Viability Monitoring: The genebank monitors the viability of the accession based on the initial viability, variety group and storage condition as follow:

a. Accessions belonging to *Oryza sativa* subsp. indica and *O. glaberrima* with an initial viability of 90-95 % are tested every five years for the active collection (AC) and every seven years for the base collection (BC); those accessions with initial viability of 96-100 %, each seven years for the AC and each 10 years for the BC.
b. For accessions belonging to *Oryza sativa* subsp. Japonica and showing an low initial viability of 85-89 %, the germination test is carried out every three years for the AC and every five years for the BC; japonica materials with 90-95 % of initial viability are tested each four years for the AC and each six years for the BC and those with 96-100 % every five years for the AC and every seven years for the BC.

Regeneration of accessions is carried out if the viability has declined to 85 % of the initial viability and when an accession in the store is less than 60 g in the active collection.

3.3 Maintaining Genetic Integrity

3.3.1 Minimum Sample Size for Regeneration: The number of seeds use for regeneration will depend on the available seeds; generally, it is 5-25 g of seeds.

3.3.2 Pollination Controls: Although rice is considered as a self-pollinating species, a study conducted by Reaño and Pham (1998) has shown that out-crossing can occur. To minimize possible out-crossing, regeneration plots at IRRI are divided with a safer distance of about 0.75 m between plots and seeds harvested only from the middle rows of each plots.

3.3.3 Appropriate Regeneration Environment: Seed regeneration for long term conservation is undertaken only in the dry season (November-May). During this season, fewer pest and diseases problems are observed, relative humidity at harvest time is lower, and pre-harvest germination of weak or non-dormant accessions is zero to minimum.

Growing diverse germplasm in a single environment presents some problems with regard to some cultural practices. Therefore, IRRI choose fields with a good irrigation and drainage system during site/area selection.

Time of planting plays an important role in seed multiplication. Factors IRRI have considered are:

a. Photosensitive materials are sown earlier, preferably during the first or second week of October so that the late vegetative stage coincides with the short days of the year, around December to February in Los Baños. Short days induce flowering of photosensitive materials. Accessions planted late are likely to remain at the vegetative stage and never flower until the following year.
b. Storage potential of japonica rice can be enhanced when grain filling coincides with the cooler environment that is prevalent in late December to early February. Therefore, early planting is also desirable.
c. If possible, areas to be used should be followed to minimize dropseed or volunteers. On the IRRI Experimental Station (ES), a fallow is practiced every other season.

3.4 Ensuring Availability

3.4.1 Policies: Germplasm is freely available on request to *bona fide* researchers in both public and private sector institutions, to NGOs, and farmers.

IRRI uses a Material Transfer Agreement (MTA) for all germplasm designated to FAO under the terms of the agreement signed in October 1994.

Under IRRI's Policy on Intellectual Property Rights, approved by the Board of Trustees in September 1994, the institute will not seek intellectual property protection on any of the germplasm it holds in trust, and provides germplasm on the understanding that a recipient of germplasm from the International Rice Genebank will not take any steps to apply intellectual property rights to these materials.

3.4.2 Seed Stock: Each accession in the base collection is made up of two

aluminum cans which are moisture resistant and rust-proof and contain 60 g of seeds each. Since seeds in the active collection are frequently retrieved and sub-sampled, three specially made re-sealable laminated aluminum foil bags (240 x 155 mm) have been used since 1992. In this case, minimal time is consumed when opening and resealing the foil bag without any additional cost. Additionally, two to five 10 g packets are also prepare to be readily available for distribution.

For wild rice species, one 50 seeds pack is prepared for the base collection and two to five pre-pack samples containing 20 seeds each for the active collection.

3.4.3 Health Status of the Accession: Incoming materials originate from direct collection by genebank staff in collaboration with national counterparts or donation from other public and private institutions, farmers and private individuals are sent to Seed Health Unit (SHU) to open the box and for post entry inspection. Additionally, all foreign incoming seeds should be accompanied by a phytosanitary certificate.

All seeds are checked by IRRI's Seed Health Unit before shipment, to ensure safe exchange of rice germplasm world wide. A Philippine phytosanitary certificate accompanies each shipment. Fumigation, hot-water and other treatments as prescribed by the Philippine Plant Quarantine and recipient authorities are undertaken.

3.4.4 Distribution Quantity: IRRI distributes germplasm on a first-come first-served basis. *Oryza sativa* and *O. glaberrima* are distributed in 10 g pre-packed samples and wild rice in packets of 20 seeds.

3.5 Providing Information

3.5.1 Genebank Management System: The data of all the rice germplasm conserved at IRRI are efficiently managed and maintained by an information system known as The International Rice Genebank Collection Information System (IRGCIS). IRGCIS is a comprehensive information system developed jointly by the staff of the Genetic Resources Center (GRC) and Computer Services (CS) at IRRI. The data are managed in Oracle8 and its application was developed in Oracle Developer2000. The system is available in a client-server environment. Oracle client software is installed in the workstation. The system is accessible to genebank staff for their daily activities. Major data (i.e. Passport, morpho-agronomic and evaluation data) of the entire collection are accessible to genebank staff thru IRRI Intranet.

3.5.2 System for Information Exchange: A data subset of all the accessions in the collection is accessible for non-IRRI staff through The System-wide Information Network for Genetic Resources (SINGER), a system that provides access to CGIAR center germplasm databases through the Internet site http://www.cgiar.org/singer. Additionally, the operations manual can be downloaded at http://www.knowledgebank.irri.org/grcOpsManual/default.htm.

4. Best Practices for Pearl Millet: An Example of ICRISAT's Approach[2]

4.1 Overview

Pearl millet may be the only cereal that reliably provides grain and fodder in dry areas. The primary importance of pearl millet lies in the particular potential to grow on shallow or sandy soil with low fertility and low water holding capacity; these attributes make pearl millet suitable to harsh environments where other crops do not grow well. In the semi-arid tropics of Asia and Africa pearl millet, together with *Sorghum*, are the most important staple food grain.

There is a need for conservation of pearl millet genetic resources due to traditional landraces have been replaced by modern, less heterogeneous high yielding cultivars and then these landraces are being lost through genetic erosion, and owing to the fact that a broad genetic base is required in order to develop new cultivars resistant to downy mildew (the most important disease of pearl millet worldwide), the parasitic witchweed (*Striga*), and several insect pests.

The International Center for Agricultural Research in the Semi-Arid Tropics (ICRISAT) was founded in 1972, and has the global research and conservation mandate for pearl millet, among others. Since its foundation, ICRISAT pursues an integrated genetic and natural resource management strategy to improve the livelihoods of the poor in semi-arid crop-livestock-tree production systems. At the present, ICRISAT genebank holds over 20,000 germplasm accession of pearl millet.

4.2 Ensuring Security

4.2.1 Physical Security

4.2.1.1 Safety Duplication: ICRISAT maintain a duplicate of all accession under the 'black-box' arrangement. Samples for safety duplication are prepared when processing seeds for long-term conservation. The safety duplicate or 'black-box' is prepared in aluminum foil packets which are vacuum sealed, containing 25 g of seeds.

4.2.1.2 Structure: *The operations manual does not provide any information about the premises*.

4.2.1.3 Equipment: *The operations manual does not provide any information about the equipments*.

4.2.1.4 Contingency Plants: *The operations manual does not provide information regarding contingency plans*.

[2] Technical information has been obtained from ICRISAT Manual of Genebank Operations and Procedures (Rao and Bramel, 2000).

4.2.2 Maintenance of Viability

4.2.2.1 Storage Conditions: The Genetic Resources Unit of ICRISAT has a short-term storage at 18-20 °C and 30-40 % of relative humidity (RH), for temporary holdings of seeds while they are dried and prepared for subsequent transfer to medium- and long-term storage. The active collection is maintained at 4 °C and 20-30 % RH and the base collection is stored at -20 °C. The seeds are dried in drying rooms and drying cabinets at 15 °C and 15 % RH.

4.2.2.2 Initial Viability: Pearl millet does not require any breaking dormancy treatment. Seeds are sowed on top of moist paper towels and place in an incubator set at 20/30 °C alternating temperature on 18/8 h duration. Evaluation of the seedlings is carried out seven days after sowing. Germination test is repeated, if the difference between the two replicates exceeds the maximum tolerance limits at 2.5 % probability.

Only materials with viability exceeding 90 % (cultivated) and 75% (wild species) are packaged for long term conservation.

4.2.2.3 Viability Monitoring: The monitoring interval depends on initial viability or in previous test and conditions of storage. Active collections of pearl millet with an initial viability exceeding 95 % are monitored every 10 years. Accessions with the initial viability between 85 % and 95 % are monitored every eight years, and those with viability lower than 85 % every five years.

Base collections with an initial viability exceeding 95 % are monitored every 20 years, those with viability between 85 % and 95 % every 15 years, and accessions with viability lower than 85 % every 10 years.

Regeneration of accessions is carried out if the viability has declined below 75 % and/or when an accession has insufficient stocks (50 g) for either distribution or conservation.

4.3 Maintaining Genetic Integrity

4.3.1 Minimum Sample Size for Regeneration: About 15 g of seeds are used per regeneration plot. Two weeks after sowing, plots are thinned to maintain a distance of 10 cm between plants within the row to provide about 120 plants per accession.

4.3.2 Pollination Controls: Pearl millet inbred lines and genetic stock are maintained by selfing. Landraces are maintained by sibbing.

Landraces

1. Cover individual panicles in parchment paper bags before stigma emergence. Staple or put a paper clip holding the corners together so that the bags are not blown off the panicle.
2. As anthers begin to dehisce, remove the bags from panicles, collect the pollen into a common paper bag, gently tapping the panicles. Cover the panicles with bags after collecting the pollen.

3. Remove the bags from panicles with stigmas emerged, dust the collected pollen on to the stigmas and cover the panicles with paper bags.

   Mark the date of pollination on the bags.

   Continue the process of pollen collection and dusting for 4-5 days in each accession, depending on panicle length and flowering duration.

   Self the plants that flower very early by covering the panicles in parchment paper bags. If the plants flower very late, pollinate them with pollen collected from tillers of the early flowering plants. If no tillers are available, self the late flowering plants too. Ensure that all plants within the accession are either sibbed or selfed.

   Remove bags two weeks after flowering (at dough stage) and clasp them around the panicles to identify sibbed panicles while harvesting.

Genetic Stocks

1. Cover individual panicles in parchment paper bags before stigma emergence.
2. Mark the date of covering on the bag.

4.3.3 Appropriate Regeneration Environment: Pearl millet regeneration is conducted in post-rainy season to facilitate flowering and seed production in photosensitive material. Alfisols (red soils) are best suited for seed regeneration. ICRISAT carries out regeneration in fields which were not under millet cultivation during previous two years to reduce risk of volunteer plants. The fields should have good drainage and be free of weeds at the time of sowing.

4.4 Ensuring Availability

4.4.1 Policies: ICRISAT has traditionally adhered to a policy of unrestricted availability of germplasm held in its genebank(s). In the interest of keeping this material available for future research and utilization, ICRISAT has undertaken, under Article 3 (b) of the Agreement with FAO, not to claim legal ownership over the designated germplasm, or to seek any intellectual property rights over that germplasm or related information. To ensure continued free availability of designated germplasm, ICRISAT has also agreed to pass on the same obligations to all future recipients of designated germplasm.

ICRISAT uses a Material Transfer Agreement (MTA) for material that was either developed by ICRISAT or was acquired prior to the entry into force of the Convention on Biological Diversity; or if it was acquired after the entering into force of the Convention on Biological Diversity, it was obtained with the understanding that it could be made available for any agricultural research, breeding and training purposes under the terms and conditions set out in the agreement between ICRISAT and FAO dated 26 October 1994.

4.4.2 Seed Stock: Accessions of pearl millet in base collection at ICRISAT consist of at least 4000 seeds, but preferably 12,000 seeds which are stored in aluminum packets. Accession size in active collection depends on the demand for accessions. Frequently requested materials can be stored in larger quantities than others. The maximum sample size held in active collection at ICRISAT is 400 g of

seeds. The active collection is stored in aluminum cans with blue cap which is used for distinguishing among the different crop.

4.4.3 Health Status of the accession: ICRISAT do not acquire imported seed, unless it is cleared by National Plant Quarantine service. Imported pearl millet seeds, must be certified as free of downy mildew. Seed materials received at National Bureau of Plant Genetic Resources (NBPGR) are subject to visual and microscopic examination. Once a pest, pathogen or weed is detected, appropriate eradication treatments such as fumigation, heat treatment or chemical dressing are given before release of the material for sowing. Pearl millet is required to be grown in Post-Entry Quarantine Isolation Area (PEQIA) to avoid possible introduction of seed-borne diseases and pests. Sowings are done under the close supervision of the Plant Quarantine Officer. Optimum number of plants is grown to harvest sufficient quantity of seeds for storage and to maintain genetic integrity of the sample.

4.4.4 Distribution Quantity: ICRISAT distributes seeds only from active or working collections. Seeds are distributed in moisture-resistant envelop and packaged in a way that they reach their destination in good condition. The standard quantity of pearl millet seed distributed per accession from ICRISAT genebank is 5 g.

4.5 Providing Information

4.5.1 Genebank Management System: ICRISAT maintain an inventory database for documentation of genebank activities. The inventory database is structured with the following type of data: (a) passport, (b) seed processing, (c) seed storage, (d) germplasm distribution (e) germplasm regeneration and (f) characterization.

4.5.2 System for Information Exchange: Information about the collection is accessible through the System-Wide Information Network on Genetic Resources (SINGER). ICRISAT has a comprehensive manual of genebank operations and procedures that can be bought at: http://www.icrisat.org/Publications/apostle/pubsearch.htm

5. Best Practices for Maize: An Example of CIMMYT's Approach[3]

5.1 Overview

Maize is believed to have originated in southern Mexico; it was the staple food of all the pre-Columbian Mesoamerican civilizations. Maize, along with wheat and rice, is one of the world's three most important cereals. Moreover, the demand for maize in developing countries is projected to surpass the demand for both wheat and rice. The crop comprises five species, *Zea mays*, *Zea luxurians*, *Zea perennis*, *Zea diploperennis* and *Zea nicaraguensis*, and 17 wild related species, all of them belonging to the genus *Tripsacum*.

The CIMMYT started activities in 1966; maize collection was based on a germplasm assemblage of the joint Rockefeller Foundation-Government of Mexico Program initiated in 1943. At that time, CIMMYT operations were oriented almost exclusively to improving crop yield. It is only until 1984 when CIMMYT decided to refit on of the chambers in order to provide long term storage for maize collection. Nowadays, CIMMYT holds over 20,000 germplasm accessions of maize and its wild relatives.

5.2 Ensuring Security

5.2.1 Physical Security

5.2.1.1 Safety Duplication: CIMMYT maintains a safety arrangement (MOU) with the USDA's National Center for Genetic Resources Preservation (NCGRP) in a cooperative manner to back-up its maize germplasm collection. At the moment, the NCGRP conserves duplicates samples of 82 % of the CIMMYT maize collection (24,450 accessions) at -20 °C, under long-term seed storage, using the identification numbers of CIMMYT genebank. Upon regeneration of accession, CIMMYT sends the NCGRP 1-2 kg seed samples to serve as a back-up. None of the seeds are distributed by NCGRP.

5.2.1.2 Structure: The Wellhausen-Anderson Genetic Resources Center facilities were built with advanced technology for medium- and long-term conservation. The genebank is a two-floor structure constructed using reinforced concrete walls. On the main floor (ground level) is a chamber maintained at -3 °C and 25-35 % relative humidity (RH) that contains the active collection.

On the lower level (below-ground) is an equivalent chamber maintained at -18 °C, here the base and black-box collections are stored. Access to the storage chambers is through a multi-locked entry system. Entry into the hallway leading to the main germplasm bank entrance is through a glass door that is opened via an electronic key card. Only authorized personnel have such a key card.

Access to the genebank anti-chamber is through steel and aluminum door with a numeric coded lock which only authorized staff have it. Inside the anti-chamber are stairs and a freight elevator leading to the lower floor and storage

[3] Technical information was obtained from CIMMYT's Operations Manual (Taba *et al*. 2004).

room. Access into each storage chamber is via a sliding steel and aluminum, thermal insulated door, with a numeric coded lock.

5.2.1.3 Equipment: Temperature and RH is monitored via remote sensing devices in several locations in both chambers. Alarms are installed to indicate when either chamber deviates from the set point. A diesel generator provides 24/7 automatic dedicated backup power to genebank lighting, air conditioning, and access lock during power outages.

5.2.1.4 Contingency Plans: *The manual does not provide information regarding emergency plans*.

5.2.2 Maintenance of Viability

5.2.2.1 Storage Conditions: CIMMYT maintain its active collection stored at -3 °C and 25-30 % of relative humidity. Seeds here have an average lifespan of approximately 30 years. The base collection is kept at -18 °C and the same relative humidity that the active collection. Seeds stored in this chamber have a shelf live of approximately 60 years. The seeds are dried in a drying room at 10 °C and 25 % of relative humidity to a seed moisture content of 6-8 %, depending upon the species, in equilibrium with drying conditions. This normally takes 6-8 weeks.

5.2.2.2 Initial Viability: The initial germination test of seed accessions must exceed 90 % germination to be stored in the genebank. Regeneration may be repeated two or three times to produce sufficient quality seed. CIMMYT follows the ISTA rule to count the seeds that have normal and abnormal germination after four days and seven days to determine percent of germination.

For maize (*Zea mays*) and teosinte, absorbent paper is used for germination test. For teosinte, the seeds can be pre-treated to break dormancy with 20 % hydrogen peroxide (H_2O_2) solution for 24 hours before germination. Some teosinte accession can germinate without seed treatment. For *Tripsacum*, the embryo is separated from rachis and germinated in aseptic conditions using N6 media without hormones. The rolled wet paper with maize seeds is placed inside a germinator at 25 °C and 100 % RH with a 12/12 hours dark/light regime. Normally two set of 50 seeds each are used for testing seed viability of each accession. If the variability between the two replications is high, another test is conducted with either 50 or 100 seeds.

5.2.2.3 Viability Monitoring: The first monitoring of seed viability is conducted after 10 years of storage in the active collection; then after every five years. In the course of seed preservation for the active collection, if seed viability of the accessions drops below 85 %, or the number of seeds falls below 1,500, the accession is regenerated.

5.3 Maintaining Genetic Integrity

5.3.1 Minimum Sample Size for Regeneration: An optimum sample size for regenerating maize landrace accessions (panmictic populations) is determined by

frequencies of the rare alleles present in the accession. Based on statistical data, CIMMYT has determined that the proper sample size is based on producing 100 or more ears for regenerating landrace accessions.

5.3.2 Pollination Controls: Artificial pollination control is made either by plant to plant crosses (dioecious mode) or by chain crosses (monoecious mode) within the accession. The appropriate mating system is used for maintaining effective population size. Plant to plant crosses requires twice as much land as chain crosses to produce the same number of ears. Usually, chain crosses are used to regenerate a large number of accessions. Inbreed lines are selfed or sib-mated. Throughout the regeneration cycle, it is important to maintain an equal effective population size to avoid genetic drift, inbreeding, and associated loss of alleles. Contamination by other germplasm or alien pollen sources must also be avoided.

5.3.3 Appropriate Regeneration Environment: Regeneration of maize germplasm accession should take place where they can adapt and reproduce themselves in a cost effective manner, while maintaining the original genetic integrity of the populations. Tropical and subtropical maize accessions are regenerated at CIMMYT's Tlaltizapan experiment station, Morelos, Mexico (940 m a.s.l.). The maize growing seasons start in October (cycle A) and April (Cycle B). Highland maize accessions are regenerated at CIMMYT's El Batan station (2,300 m a.s.l.). Andean highland and some of the Central America highland accessions are not well adapted to the El Batan highland station. They are regenerated in collaboration with the national genebanks, following the same regeneration protocol used by CIMMYT.

5.4 Ensuring Availability

5.4.1 Policies: The designated germplasm accessions (all maize landrace accessions, teosinte and *Tripsacum* in Annex 1 of the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA), and obsolete varieties and breeding populations) in the in-trust collection under CIMMYT-FAO agreement (1994) are distributed upon request to all *bona fide* users with the interim material transfer agreement (MTA) pursuant to the ITPGRFA. The germplasm accessions from CIMMYT research products are distributed with the MTA for non-designated germplasm to all *bona fide* users.

5.4.2 Seed Stock: Seed is packaged in a laminated aluminum foil packet that can contain about 1 kg (1,000-2,500 seeds) normal maize seeds. Two packets are prepared for the base collection at regeneration. For the active collection (where seed is obtained to respond to outside requests), one-gallon plastic airtight containers holding 2-3 kg (5,000-10,000 seeds) are used for storage.

5.4.3 Health Status of the Accession: All new maize germplasm (landrace collection, breeder lines, breeder populations, gene pools, genetic materials, and related species) are sent to the CIMMYT Seed Inspection and Distribution Unit (SIDU), where they are inspected following Mexican quarantine regulations in the seed laboratory and greenhouse. The SIDU works under its own operational procedures (Mezzalama *et al*., 2001).

The SIDU examines the health status of the seed for distribution. If required, the standard seed health procedure is applied to the sample accession before shipment. All shipment of genebank accessions are accompanied by the phytosanitary certificate, if necessary.

5.4.4 Distribution Quantity: Standard shipments for maize are 50-100 seeds; for teosinte 15-20 seeds; and for *Tripsacum* 5-15 seeds. The seed samples are, for the most part, prepared from active collection. The seed samples can be prepared from the base collection when the requested accessions are only preserved there and there is sufficient seed stock.

5.5 Providing Information

5.5.1 Genebank Management System: The datasets of passport, regeneration (characterization), evaluation (core subsets), seed monitoring (seed amounts, germination, storage address), and seed shipment are incorporated into the current maize genebank information database system (MZBANK). A new CIMMYT genebank database is under development.

5.5.2 System for Information Exchange: Information about the collection can be accessed through the System-Wide Information Network on Genetic Resources (SINGER). CIMMYT in order to ease users' access to germplasm collection information has produced different publications regarded with maize collection (Taba *et al*., 1999; Taba *et al*., 2004; Warburton *et al*. 2004).

6. Best Practices for Chickpea: An Example of ICRISAT's Approach[4]

6.1 Overview

Chickpea is considered the third most important food legume worldwide. It is grown in over 40 countries representing all the continents. Production and consumption are mostly made in developing countries. The species has one of the highest nutritional values of any dry edible legume; which confer critical importance in improving nourishment within developing countries.

Conservation of chickpea plant genetic resources is essential to ensure availability of basic raw materials to meet the current and future needs of crop improvement programs.

The International Center for Agricultural Research in the Semi-Arid Tropics (ICRISAT) was founded in 1972, and has the global research and conservation mandate for chickpea, among others. Since its foundation, ICRISAT pursues an integrated genetic and natural resource management strategy to improve the livelihoods of the poor in semi-arid crop-livestock-tree production systems. At the present, ICRISAT genebank holds over 15,000 germplasm accessions of chickpea.

6.2 Ensuring Security

6.2.1 Physical Security

6.2.1.1 Safety Duplication: ICRISAT maintain a duplicate of all accession under the 'black-box' arrangement. Samples for safety duplication are prepared when processing seeds for long-term conservation. The safety duplicate or 'black-box' is prepared in aluminum foil packets which are vacuum sealed, containing 100 g of seeds.

6.2.1.2 Structure: *The operations manual does not provide any information about the premises*.

6.2.1.3 Equipment: *The operations manual does not provide any information about the equipments*.

6.2.1.4 Contingency Plants: *The operations manual does not provide information regarding contingency plans*.

6.2.2 Maintenance of Viability

6.2.2.1 Storage Conditions: The Genetic Resources Unit of ICRISAT has a short-term storage at 18-20 °C and 30-40 % of relative humidity (RH), for

[4] Technical information has been obtained from ICRISAT Manual of Genebank Operations and Procedures (Rao and Bramel, 2000).

temporary holdings of seeds while they are dried and prepared for subsequent transfer to medium- and long-term storage. The active collection is maintained at 4 °C and 20-30 % RH and the base collection is stored at -20 °C. The seeds are dried in drying rooms and drying cabinets at 15 °C and 15 % RH.

6.2.2.2 Initial Viability: Only wild species of chickpea require breaking dormancy treatment. Mechanical scarification is used to break seed dormancy. The scarification is made puncturing the seed coat with a razor blade of scalpel. Seeds are sowed between moist paper towels and place in an incubator set at 20 °C. Evaluation of the seedlings is carried out seven days after sowing. After seedlings evaluation, hard and ungerminated seeds are scarified again and evaluate at 14 days after sowing. Germination test is repeated, if the difference between the two replicates exceeds the maximum tolerance limits at 2.5 % probability.

Only materials with viability exceeding 90 % (cultivated) and 75% (wild species) are packaged for long term conservation.

6.2.2.3 Viability Monitoring: The monitoring interval depends on initial viability or in previous test and conditions of storage. Active collections of chickpea with an initial viability exceeding 95 % are monitored every 10 years. Accessions with the initial viability between 85 % and 95 % are monitored every eight years, and those with viability lower than 85 % every five years.

Base collections with an initial viability exceeding 95 % are monitored every 20 years, those with viability between 85 % and 95 % every 15 years, and accessions with viability lower than 85 % every 10 years.

Regeneration of accessions is carried out if the viability has declined below 75 % and/or when an accession has insufficient stocks (100 g) for either distribution or conservation.

6.3 Maintaining Genetic Integrity

6.3.1 Minimum Sample Size for Regeneration: At least 80 plants are used for regeneration of chickpea accessions.

6.3.2 Pollination Controls: For regeneration of chickpea is not required any pollination control.

6.3.3 Appropriate Regeneration Environment: Regeneration of chickpea is carried out in the middle of October and conducted in Vertisols (black soils). The plots have good drainage and are free from weeds at the time of sowing. Land is prepared by deep ploughing, followed by 2-3 harrowings.

6.4 Ensuring Availability

6.4.1 Policies: ICRISAT has traditionally adhered to a policy of unrestricted availability of germplasm held in its genebank(s). In the interest of keeping this material available for future research and utilization, ICRISAT has undertaken, under Article 3 (b) of the Agreement with FAO, not to claim legal ownership over

the designated germplasm, or to seek any intellectual property rights over that germplasm or related information. To ensure continued free availability of designated germplasm, ICRISAT has also agreed to pass on the same obligations to all future recipients of designated germplasm.

ICRISAT uses a Material Transfer Agreement (MTA) for material that was either developed by ICRISAT or was acquired prior to the entry into force of the Convention on Biological Diversity; or if it was acquired after the entering into force of the Convention on Biological Diversity, it was obtained with the understanding that it could be made available for any agricultural research, breeding and training purposes under the terms and conditions set out in the agreement between ICRISAT and FAO dated 26 October 1994.

6.4.2 Seed Stock: Accessions of chickpea in base collection at ICRISAT consist of at least 1,000 viable seeds, but preferably 1,500-2,000 seeds which are stored in aluminum packets. Accession size in active collection depends on the demand for accessions. Frequently requested materials can be stored in larger quantities than others. The maximum sample size held in active collection at ICRISAT is 400 g of seeds. The active collection is stored in aluminum cans with yellow cap which is used for distinguishing among the different crop.

6.4.3 Health Status of the accession: ICRISAT do not acquire imported seed, unless it is cleared by National Plant Quarantine service. Imported chickpea seeds, must be certified that seed samples were collected from mother plants free of *Aschochyta rabiei* and viral diseases such as stunt and mosaic. Seed materials received at National Bureau of Plant Genetic Resources (NBPGR) are subject to visual and microscopic examination. Once a pest, pathogen or weed is detected, appropriate eradication treatments such as fumigation, heat treatment or chemical dressing are given before release of the material for sowing. Sowings are done under the close supervision of the Plant Quarantine Officer. Optimum number of plants is grown to harvest sufficient quantity of seeds for storage and to maintain genetic integrity of the sample.

6.4.4 Distribution Quantity: ICRISAT distributes seeds only from active or working collections. Seeds are distributed in moisture-resistant envelop and packaged in a way that they reach their destination in good condition. The standard quantity of chickpea seed distributed per accession from ICRISAT genebank is 100 seeds.

6.5 Providing Information

6.5.1 Genebank Management System: ICRISAT maintain an inventory database for documentation of genebank activities. The inventory database is structured with the following type of data: (a) passport, (b) seed processing, (c) seed storage, (d) germplasm distribution (e) germplasm regeneration and (f) characterization.

6.5.2 System for Information Exchange: Information about the collection can be accessed through the System-Wide Information Network on Genetic Resources (SINGER). ICRISAT has a comprehensive manual of genebank operations and

procedures that can be bought at: http://www.icrisat.org/Publications/apostle/pubsearch.htm

7. Best Practices for Wheat: An Example of CIMMYT's Approach[5]

7.1 Overview

Wheat is one of the major cereal staples; it is the most widely grown on the earth. The crop is grown from Southern Chile to Norway and from sea level to 4,000 m a.s.l. Wheat is a major staple and calorie source for more than half of the world population.

The rapid release of new cultivars to farmers, particularly in developing countries, and the replacement of local landraces represent a threat to the genetic diversity of wheat. For these reasons, it is worthy to conserve these genetic resources, in order to use this genetic base to meet current and future needs of crop improvement programs.

The CIMMYT started activities in 1966. However, CIMMYT operated a relatively small germplasm cold storage facility for conserving small amounts of certain wheat genetic material. It is only until 1981 when the first actual genebank of limited capacity became operational. Nowadays, CIMMYT holds over 60,000 germplasm accessions of wheat.

7.2 Ensuring Security

7.2.1 Physical Security

7.2.1.1 Safety Duplication: CIMMYT maintains agreements with the USDA's National Center for Genetic Resources Preservation (NCGRP) and International Center for Agricultural Research in the Dry Areas (ICARDA) to send these two parties safety duplications of CIMMYT wheat and wheat-related accessions. CIMMYT sends the NCGRP and ICARDA seed samples containing 10 g, to serve as a back-up. The seeds are distributed neither by NCGRP nor by ICARDA.

7.2.1.2 Structure: The Wellhausen-Anderson Genetic Resources Center facilities were built with advanced technology for medium- and long-term conservation. The genebank is a two-floor structure constructed using reinforced concrete walls. On the main floor (ground level) is a chamber maintained at -3 °C and 25-35 % relative humidity (RH) that contains the active collection.

On the lower level (below-ground) is an equivalent chamber maintained at -18 °C, here the base and black-box collections are stored. Access to the storage chambers is through a multi-locked entry system. Entry into the hallway leading to the main germplasm bank entrance is through a glass door that is opened via an electronic key card. Only authorized personnel have such a key card.

Access to the genebank anti-chamber is through steel and aluminum door with a numeric coded lock which only authorized staff have it. Inside the anti-chamber are stairs and a freight elevator leading to the lower floor and storage room. Access into each storage chamber is via a sliding steel and aluminum, thermal insulated door, with a numeric coded lock.

[5] Technical information was obtained from CIMMYT's Operations Manual (Taba *et al*. 2004).

7.2.1.3 Equipment: Temperature and RH is monitored via remote sensing devices in several locations in both chambers. Alarms are installed to indicate when either chamber deviates from the set point. A diesel generator provides 24/7 automatic dedicated backup power to genebank lighting, air conditioning, and access lock during power outages.

7.2.1.4 Contingency Plans: *The manual does not provide information regarding emergency plans*.

7.2.2 Maintenance of Viability

7.2.2.1 Storage Conditions: CIMMYT maintain its active collection stored at -3 °C and 25-30 % of relative humidity. Seeds here have an average lifespan of approximately 30-50 years. The base collection is kept at -18 °C and the same relative humidity that the active collection. Seeds stored in this chamber have a shelf live of approximately 60 years. The seeds are dried in a drying room at 10 °C and 25 % of relative humidity to a seed moisture content of 5-7 %, depending upon the species, in equilibrium with drying conditions. This normally takes 6-8 weeks.

7.2.2.2 Initial Viability: The initial germination test of seed accessions must exceed 90 % germination to be stored in the genebank. Regeneration may be repeated two or three times to produce sufficient quality seed. CIMMYT follows the ISTA rule to count the seeds that have normal and abnormal germination after four days and seven days to determine percent of germination.

The Petri dishes with wheat seeds is placed inside a germinator at 25 °C and 100 % RH with a 12/12 hours dark/light regime. Normally two set of 50 seeds each are used for testing seed viability of each accession. If the variability between the two replications is high, another test is conducted with either 50 or 100 seeds.

7.2.2.3 Viability Monitoring: The first monitoring of seed viability is conducted after 10 years of storage in the active collection; then after every five years. In the course of seed preservation for the active collection, if seed viability of the accessions drops below 85 %, or the number of seeds falls below 1,500, the accession is regenerated.

7.3 Maintaining Genetic Integrity

7.3.1 Minimum Sample Size for Regeneration: The sample size used for regenerating wheat accessions is 25-50 seeds.

7.3.2 Pollination Controls: For regeneration of wheat is not required any pollination control.

7.3.3 Appropriate Regeneration Environment: CIMMYT regenerates wheat seed in a disease free location or within a semi-contained screenhouse, to obtain healthy seed prior cooled storage. Depending on whether the accession is a spring or winter habit genotype, or a domesticated or wild relative they are

regenerated in three different locations:

a. Mexicali is located in the northeast of Mexico, where Karnal bunt (KB), a quarantinable disease does not occur. Here, domesticated spring wheat accessions are regenerated. During flowering time, when in theory KB spores could infect florets; fungicides are applied at intervals to cover all accessions, even if they differ in flowering date.
b. Toluca is located in the Mexican central highlands at 2,640 m a.s.l., and in winter experiences at least eight weeks of temperatures below 4 °C at night. Generally these conditions are sufficient to vernalize most facultative and winter wheat accessions. Winter wheat accessions are regenerated here, if their day length requirement is not too long. They are also treated with fungicides as described above.
c. Screenhouses at El Batan, CIMMYT, HQ. This location is also located in the Mexicali central highlands, though 400 meters lower than Toluca. The screenhouses are equipped with special high-intensity lighting, to allow the long day-length required by day-length sensitive wheat accessions to be satisfied.

7.4 Ensuring Availability

7.4.1 Policies: The designated germplasm accessions in the in-trust collection under CIMMYT-FAO agreement (1994) are distributed upon request to all *bona fide* users with the interim material transfer agreement (MTA) pursuant to the ITPGRFA. The germplasm accessions from CIMMYT research products are distributed with the MTA for non-designated germplasm to all *bona fide* users.

7.4.2 Seed Stock: Seed is packaged in a laminated aluminum foil packet that can contain about 100 g (about 3,000) wheat seeds. One packet is prepared for the base collection at regeneration. For the active wheat collection, laminated foil packets holding 250 grams (about 7,000 seeds) are used.

7.4.3 Health Status of the Accession: All new wheat germplasm (landrace collection, breeder lines, breeder populations, gene pools, genetic materials, and related species) are sent to the CIMMYT Seed Inspection and Distribution Unit (SIDU), where they are inspected following Mexican quarantine regulations in the seed laboratory and greenhouse. The SIDU works under its own operational procedures (Mezzalama *et al*., 2001).

The seed is treated with fungicide / insecticidal mixture just prior to shipment in order to strictly adhere to official regulations of national governments regarding seed imports of recipient cooperators.

7.4.4 Distribution Quantity: Standard shipments for wheat are 50-100 seeds. The seed samples are, for the most part, prepared from active collection. The seed samples can be prepared from the base collection when the requested accessions are only preserved there and there is sufficient seed stock.

7.5 Providing Information

7.5.1 Genebank Management System: Presently, the Wheat Germplasm Bank System (WGBS) is being used for data management. This is a component of the IWIS software system that electronically manages data (i.e. passport, characterization, evaluation, and logistical information) in the germplasm bank collection. WGBS can also generate field books and data reports.

7.5.2 System for Information Exchange: Information about the collection can be accessed through the System-Wide Information Network on Genetic Resources (SINGER).

8. Best Practices for Pigeonpea: An Example of ICRISAT's Approach[6]

8.1 Overview

Pigeonpea ranks sixth in area and production in comparison to other grain legumes such as beans, peas, and chickpeas. Pigeonpea represents about 5% of the total world production of pulses. It is grown in over 50 countries in Asia, Africa and America. Production and consumption are mostly made in developing countries.

Conservation of Pigeonpea plant genetic resources is essential to ensure availability of basic raw materials to meet the current and future needs of crop improvement programs.

The International Center for Agricultural Research in the Semi-Arid Tropics (ICRISAT) was founded in 1972, and has the global research and conservation mandate for pigeopea, among others. Since its foundation, ICRISAT pursues an integrated genetic and natural resource management strategy to improve livelihoods of the poor in semi-arid crop-livestock-tree production systems. At the present, ICRISAT genebank holds over 13,000 germplasm accessions of pigeonpea.

8.2 Ensuring Security

8.2.1 Physical Security

8.2.1.1 Safety Duplication: ICRISAT maintain a duplicate of all accession under the 'black-box' arrangement. Samples for safety duplication are prepared when processing seeds for long-term conservation. The safety duplicate or 'black-box' is prepared in aluminum foil packets which are vacuum sealed, containing 100 g of seeds.

8.2.1.2 Structure: *The operations manual does not provide any information about the premises*.

8.2.1.3 Equipment: *The operations manual does not provide any information about the equipments*.

8.2.1.4 Contingency Plants: *The operations manual does not provide information regarding contingency plans*.

8.2.2 Maintenance of Viability

8.2.2.1 Storage Conditions: The Genetic Resources Unit of ICRISAT has a short-term storage at 18-20 °C and 30-40 % of relative humidity (RH), for

[6] Technical information has been obtained from ICRISAT Manual of Genebank Operations and Procedures (Rao and Bramel, 2000).

temporary holdings of seeds while they are dried and prepared for subsequent transfer to medium- and long-term storage. The active collection is maintained at 4 °C and 20-30 % RH and the base collection is stored at -20 °C. The seeds are dried in drying rooms and drying cabinets at 15 °C and 15 % RH.

8.2.2.2 Initial Viability: Only wild species of pigeonpea require breaking dormancy treatment. Mechanical scarification is used to break seed dormancy. The scarification is made puncturing the seed coat with a razor blade of scalpel. Seeds are sowed between moist paper towels and place in an incubator set at 25 °C. Evaluation of the seedlings is carried out seven days after sowing. After seedlings evaluation, hard and ungerminated seeds are scarified again and evaluate at 14 days after sowing. Germination test is repeated, if the difference between the two replicates exceeds the maximum tolerance limits at 2.5 % probability.

Only materials with viability exceeding 90 % (cultivated) and 75% (wild species) are packaged for long term conservation.

8.2.2.3 Viability Monitoring: The monitoring interval depends on initial viability or in previous test and conditions of storage. Active collections of pigeonpea with an initial viability exceeding 95 % are monitored every 10 years. Accessions with the initial viability between 85 % and 95 % are monitored every eight years, and those with viability lower than 85 % every five years.

Base collections with an initial viability exceeding 95 % are monitored every 20 years, those with viability between 85 % and 95 % every 15 years, and accessions with viability lower than 85 % every 10 years.

Regeneration of accessions is carried out if the viability has declined below 75 % and/or when an accession has insufficient stocks (100 g) for either distribution or conservation.

8.3 Maintaining Genetic Integrity

8.3.1 Minimum Sample Size for Regeneration: A minimum of 180 plants are used for regeneration of pigeonpea accessions.

8.3.2 Pollination Controls: Pigeonpea is cross-pollinating (0-40 %, depending on genotype and insect pollinator populations). Seed regeneration must preclude cross pollination.

Cover at least 180 plants in muslin cloth bags or in pollination cages before flowering.

Spray Thiodan (@ 2mL/L) before covering the plants.

When small number of cultivars is to be regenerated for large-scale seed production, geographic isolation of about 100 m is desirable.

8.3.3 Appropriate Regeneration Environment: Regeneration of pigeonpea is conducted in Vertisols (black soils). Late sowing results in reduced plant height, and thus allows whole plants to be covered with muslin cloth bags. Soil tests should be carried out prior sowing to ensure satisfactory fertility.

8.4 Ensuring Availability

8.4.1 Policies: ICRISAT has traditionally adhered to a policy of unrestricted availability of germplasm held in its genebank(s). In the interest of keeping this material available for future research and utilization, ICRISAT has undertaken, under Article 3 (b) of the Agreement with FAO, not to claim legal ownership over the designated germplasm, or to seek any intellectual property rights over that germplasm or related information. To ensure continued free availability of designated germplasm, ICRISAT has also agreed to pass on the same obligations to all future recipients of designated germplasm.

ICRISAT uses a Material Transfer Agreement (MTA) for material that was either developed by ICRISAT or was acquired prior to the entry into force of the Convention on Biological Diversity; or if it was acquired after the entering into force of the Convention on Biological Diversity, it was obtained with the understanding that it could be made available for any agricultural research, breeding and training purposes under the terms and conditions set out in the agreement between ICRISAT and FAO dated 26 October 1994.

8.4.2 Seed Stock: Accessions of pigeonpea in base collection at ICRISAT consist of at least 4000 seeds, but preferably 12,000 seeds which are stored in aluminum packets. Accession size in active collection depends on the demand for accessions. Frequently requested materials can be stored in larger quantities than others. The maximum sample size held in active collection at ICRISAT is 400 g of seeds. The active collection is stored in aluminum cans with green cap which is used for distinguishing among the different crop.

8.4.3 Health Status of the Accession: ICRISAT do not acquire imported seed, unless it is cleared by National Plant Quarantine service. Seed materials received at National Bureau of Plant Genetic Resources (NBPGR) are subject to visual and microscopic examination. Once a pest, pathogen or weed is detected, appropriate eradication treatments such as fumigation, heat treatment or chemical dressing are given before release of the material for sowing. Pigeonpea is required to be grown in Post-Entry Quarantine Isolation Area (PEQIA) to avoid possible introduction of seed-borne diseases and pests. Sowings are done under the close supervision of the Plant Quarantine Officer. Optimum number of plants is grown to harvest sufficient quantity of seeds for storage and to maintain genetic integrity of the sample.

8.4.4 Distribution Quantity: ICRISAT distributes seeds only from active or working collections. Seeds are distributed in moisture-resistant envelop and packaged in a way that they reach their destination in good condition. The standard quantity of pigeonpea seed distributed per accession from ICRISAT genebank is 200 seeds.

8.5 Providing Information

8.5.1 Genebank Management System: ICRISAT maintain an inventory database for documentation of genebank activities. The inventory database is structured

with the following type of data: (a) passport, (b) seed processing, (c) seed storage, (d) germplasm distribution (e) germplasm regeneration and (f) characterization.

8.5.2 System for Information Exchange: Information about the collection can be accessed through the System-Wide Information Network on Genetic Resources (SINGER). ICRISAT has a comprehensive manual of genebank operations and procedures that can be bought at: http://www.icrisat.org/Publications/apostle/pubsearch.htm

9. Generic *in vitro* Genebank Standards

9.1 Overview

The feasibility of using *in vitro* culture methods for plant genetic resources conservation was recognized during the late 1970s (IPGRI/CIAT, 1994). The International Board for Plant Genetic Resources (IBPGR) acknowledged, in the early 1980s, that the state of knowledge of *in vitro* culture methods was insufficient and had many limitations for conserving genetic resources; therefore, more scientific research should be addressed to this area (Withers, 1980; IBPGR, 1983). At present, *in vitro* methods are playing a more important role in the conservation of plant genetic resources, especially for vegetatively propagated and 'recalcitrant' seed producing species, germplasm that can only be conserved in field genebanks. *In vitro* methods have also an important role in the safe movement of germplasm due to the production of disease free planting materials as well as an "intermediary step" in cryopreserving germplasm.

Currently, increasing number of field genebanks have developed *in vitro* active collections for clonally propagated species, e.g. the cassava collection at CIAT (IPGRI/CIAT, 1994), the yam collection at IITA (Ng and Ng, 1999), the temperate fruit and nut crops collection at the US NCGR-Corvallis (Reed, 1999), the *Allium* at IPK-Gatersleben (Keller, 1991a, b), etc. Additionally, at the *Musa* Germplasm Transit Center of INIBAP the global banana collection was established (Van den Houwe, 1999). Nevertheless, these developments took place without the benefit of generic *in vitro* genebank standards being available. This lack of standards hampers the harmonization of the global genebank network and difficult networking. Literature regarding *in vitro* collection management is scarce and not compiled in a comprehensive manner. IPGRI attempted to fill this gap and produced a status report on the development and application of *in vitro* techniques (Ashmore, 1997); and more recently, a technical guideline for the management of field and *in vitro* collections (Reed *et al*., 2004); however, there is still the necessity to produce generic genebank standards for *in vitro* collection management in order to harmonize genebank management.

The present generic *in vitro* genebank standards refer only to growing *in vitro* collections; cryopreserved collections are excluded in order to simplify the setting of the standards.

9.2 Ensuring Security

It refers to the security of the genebank structure itself (i.e. its physical security) and to the safety of its germplasm (i.e. the maintenance of viability) which together will ensure the safe conservation of the entire collection.

9.2.1 Physical Security

To ensure the physical security of the collections, these *in vitro* generic standards are regarded as essential prerequisite for achieving the objective:

9.2.1.1 Safety Duplication: Duplication is required to ensure that accessions conserved in the genebank are safely preserved. If the *in vitro* collection is the only source of germplasm, a duplicate should be stored in at least another place which can be under 'black-box' arrangement or in active storage. On-site duplications

should be avoided due to the risks of losing both collections. If cryopreserved material is available, it may also serve as safety duplication.

9.2.1.2 Structure: The premises should be built to withstand most of likely natural disasters like hurricanes, cyclones, earthquakes, flooding, etc. The storage facilities should also be protected with fences, alarm system, security doors and any other systems that help to shield the genebank against burglars. Additionally, an early detection fire system should be in place and the premises should be equipped with fire isolating doors and extinguisher equipment.

9.2.1.3 Equipment: The genebank should be equipped with an emergency electrical generator which provides automatically back-up power to the storage rooms, essential genebank lighting, monitoring devices, and access locks during electrical power failures. Monitoring devices for temperature and relative humidity should be available in the storage rooms to track the actual parameters against time. The growth room should have a High Efficiency Particle-removal Air system (HEPA). It is also recommended that the rooms are provided with a back-up heat/cooling system which will keep the right temperature in case of main system fails. Electrical generator, air conditioning and any other back-up systems must be run alternately on a regular time basis to ensure that they continue to work properly. Filters of the building ventilation system, if any, should be routinely changed. Since cleanliness is critically important in tissue culture laboratories, facilities and equipments should be carefully cleaned on a regular time basis.

9.2.1.4 Contingency Plans: An emergency plan for the genebank should be implemented; it ought to describe actions and measures to be taken when any possible disaster occurs; in addition, genebank staff should be trained to confront any emergency situation, especially to face power failure emergencies. A full risk assessment of the genebank operation (identifying sources of risk and desired outcomes) should be carried out and published.

9.2.2 Maintenance of Viability

It refers to the maintenance of the highest quality of the germplasm in storage. The quality of the explants is an important condition for ensuring the longest lifespan of the accessions and hence maximum efforts need to be taken to maintain the germplasm placed in storage with the highest possible quality. The following five generic standards are required:

9.2.2.1 Normal-growth Storage Conditions: Regulation of the relative humidity (RH) is difficult in most laboratories; however, genebank should aim to control it between 40-50 % RH, a higher humidity will increase fungal growth and lower will desiccate cultures and create dust problems. Light requirement range from 10 to 1,000 µmol s^{-1} m^{-2} but most plant cultures require 50-200 µmol s^{-1} m^{-2}, with a photoperiod of 12-16 hours per day; nevertheless, the appropriate levels will depend upon the species in question. Storage temperature will depend upon the requirements of the species, in particular whether or not of tropical origin, common growth room temperatures range from 22 to 28 °C.

9.2.2.2 Slow-growth Storage Conditions: There are three methods for reducing *in vitro* growth rates which are physical (reduced temperature and light conditions),

chemical (using growth retardants) and a combination of the two. Temperature will vary upon species stored. Temperate crops may be stored at 4 °C and tropical ones require 15-20 °C. Light conditions may be darkness or a 12-16 h photoperiod, the light intensity required will vary from species to species. The humidity should be between 40-50 % to avoid problems linked to humidity.

9.2.2.3 Initial Quality of the Explants: Recognizing that the initial quality of the explants has a direct influence on the germplasm lifespan in the storage, the target to achieve will be the highest possible quality. The criteria for a good quality explants are: (1) normal, true to type mother plant, (2) vigorous, and (3) pest and disease free. Since tissue culture vigor directly reflects the state of the mother plant, all plant material selected for tissue culture should be collected from vigorous and healthy mother plants.

9.2.2.4 Accessions Monitoring: Plants in slow-growth storage should be monitored every 1-3 months, depending upon the species stored, to assess their viability. A visual assessment should be carried out to corroborate that the plants are still viable and with good vigor; additionally, depending upon species, other criteria should be taken into account like absence of necrosis, chlorosis, hyperhidricity, blackening, contamination, callus formation and defoliation (Van den Houwe, 1999). Also the accessions should be monitored at the same interval to assess their need for re-culturing. Accessions must be sub-cultured if the number of replicates has been reduced to 3-12 which depends on species stored and purpose of the collection (e.g. active distribution, conservation, etc.) or if the quality has drastically decreased.

9.2.2.5 Subculture Practices: Genebank should develop and establish a routine for subculturing of the replicates. This routine should be as secured as possible, in order to guarantee that the accession is not being lost as a result of human handling and/or contamination.

9.3 Maintaining Genetic Integrity

Tissue culture conditions entail the risk of inducing/enhancing the prospect of genetic changes (i.e. somaclonal variation). To achieve the objective of maintaining the genetic integrity of the sample, the stored germplasm should be visually monitored (see below), on regular intervals, to verify the genetic stability of the material.

9.3.1 Monitoring Genetic Stability: Plants should be monitored to verify if any somaclonal variation occurs. The genebank should examine visually the germplasm at regular time intervals and in case that any abnormality is found, the tissue culture plants must be grown for an entire cycle in field or greenhouse to observe for any changes in morphology. Initial characterization of the accession is essential for comparison. Since the technique of visual examination of the *in vitro* plants is not very reliable, germplasm that has been kept in storage for long time and/or sub-cultured many times should be grown in field to verify the characteristics of the accession, depending upon the species stored. Those genotypes that are observed to be prone to somaclonal variation should be selected randomly for testing.

9.4 Ensuring Availability

An important objective of conservation efforts is to facilitate the effective utilization of germplasm accessions by researchers, breeders and farmers. Thus, ensuring the ready availability of stored germplasm is an important principle. It refers to the ability of genebanks to supply and distribute the stored germplasm, together with any associated information, to users. Aspects that can affect the availability include: (a) policies, (b) germplasm stock, (c) health status of accessions, and (d) distribution quantity.

9.4.1 Policies: The availability of germplasm accessions will depend on the legal status of the individual germplasm accessions. There are four basic categories of germplasm in relation to its legal status:

a. Accessions of crops and crops complexes that are listed in Annex 1 of the International Treaty (IT) of PGRFA is governed by the Treaty; access to these genetic resources will be provided under a standard material transfer agreement (sMTA) approved by the IT's Governing Body,
b. Accessions that have not been listed in Annex 1 of the IT and have been acquired after the entering into force of the Convention on Biological Diversity at January 1st, 1994. Access to these genetic resources will be provided imposing on the user the terms and conditions set by the country of origin of said resources (which could match those of the IT or even be no conditions),
c. All other genetic resources, that have not been listed in Annex 1 of the IT and have been acquired before January 1st, 1994, which the CGIAR hopes to make available under terms and conditions identical to those stated in the standard MTA of the IT (this is subject to the approval of the Governing Body of the IT) and,
d. Material that is improved or is under development, which the CGIAR also hopes to make available under conditions that are the same as, or at least broadly similar to, those of the sMTA.

For further guidance on germplasm exchange policies, please refer to Fowler et al. (2003) or Barton and Siebeck (1994).

9.4.2 Germplasm Stock: Genebanks should ensure to keep at all times adequate amounts of replicates for each accession in order to be able to supply to requestors and carry out re-culturing. It is difficult to set a generic standard for the number of plants that should be conserved for all genebanks; this will depend on the purpose of the genebank (active distribution/medium-term storage) and the risk of loss. It is necessary to determine the number of plants that are lost during a given period of time. As a minimum generic standard, five plants should be kept when the objective of the genebank is conservation.

9.4.3 Health Status of Accession: Spread of diseases (particularly viruses) through accessions may decrease the ultimate availability of the germplasm material. Accessions that are being distributed should be pest and disease free (at least of quarantine pests and diseases) in order to comply with national and international quarantine regulations. Therefore, collection managers should aim to

store tissue cultures from plants that are disease free, whenever possible. Virus indexing should be carried out on all new in-coming accessions and eventually virus eradication treatment applied. Accessions known to have viruses should not be distributed until virus eradication treatments have successfully been applied.

For further information about crop specific protocols for germplasm movement, refer to the IPGRI/FAO's "Technical Guidelines for the Safe Movement of Germplasm" which can be downloaded at:
http://www.ipgri.cgiar.org/system/page.asp?frame=publications/indexpub.htm

9.4.4 Distribution Quantity: It is recommended that genebank managers distribute germplasm samples with an adequate number of plants per accession and with good vigor to enable end-users to sub-culture the requested accession. As a minimum generic standard, it is recommended to provide at least three *in vitro* plants per accession.

9.5 Providing Information

The lack of adequate information on a given accession may well decrease the value of that accession to the end-user. The information on individual accessions should be as complete as possible in order to contribute to the effective conservation and use of genetic resources. This applies for example in the identification of duplicates and/or the selection of accessions with desirable characteristics/traits. To achieve this, a genebank should have a management system in place that allows optimizing management of the collections as well as providing access to valuable information about the material to end-users (e.g. breeders, researchers, etc.).

9.5.1 Genebank Management System: The daily management of a genebank requires access to accurate information on the germplasm conserved. These operations cover a wide range of activities from acquisition, registration, germplasm health, regeneration, evaluation as well as distribution to end-users. To achieve this, the system should allow staff members to access information about the following types of data: (a) passport, (b) management, (c) field and *in vitro* characterization, (d) evaluation, (e) mode of reproduction, and (f) germplasm distribution. Such management system differs a lot from a conventional information system that attempts to capture and manage information. A genebank management system requires an effective decision-making system based on daily operation workflows.

9.5.2 System for Information Exchange: In order to facilitate access and use of germplasm by the end-users, it is required that the most valuable information on the origin, characteristics and performance of the material is made readily available. Establishing such information exchange mechanism first requires a better understanding of the potential users and their needs. The management system should be structured taking into consideration the following aspects:

a. It should be developed taking into consideration the needs of the possible different users (e.g. farmers, researchers, breeders, etc.).

b. Genebank knowledge about the collection should be included in the management system (e.g. breaking dormancy methods, important traits, recommended subsets for particular traits combination, etc.).
c. The system should be able to monitor germplasm flow and to obtain users feedback.

The easiest way to give users access to the relevant information may be through the Internet. However, it should not be forgotten that access to the Internet is not reliable in some countries or not available at all; for these reasons, critical information of genebank operations should be also published in germplasm catalogs, articles, publications, or operations manuals.

10. Best Practices for *Musa*: An Example of INIBAP-Transit Center's[7] Approach[8]

10.1 Overview

Banana originates in Asia and the Pacific but nowadays, the crop is spread and widely grown throughout all tropical and subtropical regions. Bananas and plantains are an essential staple food for more than 400 millions of people in Asia, Africa, South and Central America and the Caribbean. Bananas are of major global importance in terms of food and income security to millions of smallholder farmers in developing countries.

Since vegetatively propagated materials enhance the incidence of disseminating diseases, one of the major advantages of *in vitro* conservation of *Musa* genetic resources is that tissue culture collections are a source of disease-free material. These collections shall be the genetic base for current and future breeding programs and a source of safety material for distribution to researchers.

The Transit Center of INIBAP was founded in 1984 which the mission of safeguarding *Musa*'s genetic diversity. The ITC was established in Belgium where no bananas grown. The absence of banana diseases in the hosting country provides a safe storage and secure basis for further distribution of banana diversity. At present, the ITC holds the world's largest collection of Musa.

10.2 Ensuring Security

10.2.1 Physical Security

10.2.1.1 Safety Duplication: At present, the collection is being backed-up in liquid nitrogen. Three cryopreservation methods are applied to freeze the different banana genotypes available in the collection. Actually, 35% of the collection is cryopreserved. As an additional safety measure, replicates of these cryopreserved accessions will be stored off-site. These replicates are yet available.

10.2.1.2 Structure: The building is designed to withstand environmental risks to certain extent. Nevertheless, the collection is hosted in a country of low environmental risks. The collection room is always locked and only genebank staffs have access to the storage room. Additionally, the building is under surveillance of a private security service hired by the hosting institution. Automatic fire and gas alarm systems are installed. The building is equipped with extinguishers, following Belgian safety regulations.

10.2.1.3 Equipment: The genebank does not have an emergency generator; nevertheless, the storage room was designed to maintain a constant temperature during several hours, when power fails. Culture and storage rooms are equipped with temperature remote sensing devices. Relative humidity is measured at regular time intervals. A High Efficient Particle-removal Air system (HEPA) is

[7] The acronym stands for International Network for the Improvement of Banana and Plantain-International Transit Center.

[8] The technical information has been obtained from Dr. Ines Van den houwe, Head of the INIBAP-ITC.

installed in the genebank. Culture rooms are provided with individual pre-settable heat/cooling units.

Heating/cooling units, laminar air-flows and autoclaves are checked for technical failures on yearly basis. Most laboratory equipment is cleaned several times every year, to reduce the risk of spreading contamination.

10.2.1.4 Contingency Plans: ITC has an emergency plan describing actions and measures to be taken when a disaster occurs. Genebank staffs are trained to confront any emergency situation. Genebank procedures and protocols are described in a guide for internal use.

10.2.2 Maintenance of Viability

10.2.2.1 Normal-growth Storage Conditions: Temperature in storage room is maintained at around 28 °C (±2 °C) with a relative humidity of 40 %. Light intensity is regulated at 63 µmol/m²/s, using a photoperiod of 24/24 h light/darkness.

10.2.2.2 Slow-growth Storage Conditions: Temperature in the storage room is maintained at 16 °C (±1 °C) with a relative humidity of 75 %. Light intensity is regulated at 25 µmol/m^2/s, with a photoperiod of 24/24 h light/darkness.

10.2.2.3 Initial Quality of the Explants: Tissue cultures that are maintained in the collection are initially derived from young shoots collected from a mother plant which is: (a) found free from banana pests and diseases after a visual inspection, (b) growing vigorously in the field, and (c) fully described (preferably) for its morphological and taxonomic characters in the field.

10.2.2.4 Accessions Monitoring: *In vitro* accessions are visually checked on a monthly basis. The parameters used for evaluating the plants are numbers of viable cultures, contamination (bacteria and fungi), and occurrence of blackening, chlorosis and necrosis.

When the number of viable cultures within the accession drops below 12, the accession is removed from storage and subcultured into a new set of 20 fresh tissue cultures.

Accessions which do not meet the qualitative criteria are removed from the storage and subcultured into a new set of 20 fresh tissue cultures.

10.2.2.5 Subculture Practices: Annual subculturing of the replicates is carried out in a two step process. Where an operator starts to subculture half of the replicates, another different operator will continue subculturing the remaining cultures of the accession. Alternatively, the annual subculturing of the accession can be completed by the same operator but on a different moment (preferably not the same day). This procedure ensures that germplasm accessions are not lost as a consequence of accidental contamination due to human handling or eventual environment contamination.

10.3 Maintaining Genetic Integrity

10.3.1 Monitoring Genetic Stability: *In vitro*: plants are visually checked on a monthly basis. In case of any abnormal growth is found, the accession is

regenerated and transferred to the greenhouse for observation of the morphological characters during the vegetative growing phase.

In vivo : based on the time period that the plant material of the accession has been kept continuously *in vitro* (number of storage cycles and/or number of years kept *in vitro*), accessions are regenerated and observed in field during at least two subsequent growing cycles. Morphological and taxonomic characteristics of the plants are compared with those of the original accession. The criteria for regeneration are: (a) 10 or more storage cycles, and/or (b) 10 or more years kept *in vitro*.

10.4 Ensuring Availability

10.4.1 Policies: Germplasm is provided to users under the terms and conditions of a material transfer agreement (MTA), which ensures that the genetic resource and related information, stays in the public domain. Two different MTAs are used by INIBAP: (a) MTA for germplasm cover by INIBAP/FAO agreement (http://www.inibap.org/pdf/mta1_en.pdf) and (b) MTA for the transfer of improved varieties of *Musa* (http://www.inibap.org/pdf/mta2_en.pdf).

10.4.2 Germplasm Stock: In the medium term collection, 20 replicates per accession are maintained. Additional stocks (usually 24-48 cultures) are maintained under normal growth conditions, for those accessions that are frequently distributed.

10.4.3 Health Status of Accession: Virus indexing is carried out on all new in-coming accessions and eventually virus eradication treatments are applied. Accessions known to have viruses are not distributed until virus eradication treatments have successfully been applied. Germplasm samples are distributed as virus-indexed tissue cultures, in compliance with FAO/IPGRI technical guidelines for safe movement of germplasm and following national and internationals quarantine regulations.

10.4.4 Distribution Quantity: ITC distributes five samples per accession, as proliferating tissues or rooted plants.

10.5 Providing Information

10.5.1 Genebank Management System: Recently, a tailored management system has been put in place, *Musa* Gene Bank Management System (MGBMS). The database is searchable by the gene bank curator and staff for specific information through a range of queries.

As part of the system, bar-coding has been introduced in the genebank. A mobile device with integrated bar-code reader allows capturing and retrieving information on the spot by genebank staff. The ITC genebank management system is linked up with the INIBAP information system.

MGBMS manages information related to accessions and it processes all data generated by different genebank operations.

The system creates an identifier for each accession (ITC code) and handles the passport data, its designation status and taxonomic information. It keeps record of genebank operation data, including storage location, stocks, monitoring,

health tests, and the distribution status. The system also manages germplasm orders, shipment related information and files genebank 'contacts' information.

10.5.2 System for Information Exchange: Users have access through the *Musa* Germplasm Information System (MGIS). This system manages passports, management data (i.e. germplasm distribution information), *in vitro* and field characterization and evaluation data (including photographs of the plants and molecular characterization) and GIS information on accessions in 18 *Musa* collections around th world, including the INIBAP-ITC collection. The database can be queried using accession number, origin, characteristics and distribution of individual accessions in the collections.

The database is accessible for users worldwide through internet (http://mgis.grinfo.net/). The database is also available on CD-Rom for users who do not have access to internet. Catalogues (*Musa*logue) of *Musa* diversity are produced, which contain the main morphological and taxonomic characteristics and photographs of wild and cultivated bananas. These catalogues are available as books, CD or can be accessed through the INIBAP website.

The data of the INIBAP-ITC collection, which are part of the MGIS database, are also available through the System-Wide Information Network on Genetic Resources (SINGER).

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